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Research Report

Role of circulating androgen levels in effects of apoE4 on cognitive function

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Abstract

Compared with apoE2 and E3, apoE4 increases the risk of cognitive impairments and of developing Alzheimer's disease (AD). ApoE4 interacts with female sex, further increasing AD risk. Previously, we showed that female *Apoe*$^{-/-}$ mice are more susceptible to apoE4-induced cognitive deficits than male mice. Androgens protect against these deficits and apoE4 male mice are more sensitive to acute blockade of androgen receptors than apoE3 male mice. To determine the chronic effects of reduced circulating androgen levels on susceptibility to the effects of apoE4 on cognitive function in males, we castrated and sham-castrated apoE4, apoE3, and *Apoe*$^{-/-}$ male mice and behaviorally compared them 3 months later. Castration impaired novel location recognition in apoE4, but not apoE3 or *Apoe*$^{-/-}$, mice. In contrast, castration impaired novel object recognition and spatial memory retention in the water maze in *Apoe*$^{-/-}$, but not apoE3 or apoE4, mice. On the contrary, castrated, but not sham-castrated, apoE4 mice showed improved acquisition over the first two hidden platform sessions and spatial memory retention in the first probe trial. While apoE3 and *Apoe*$^{-/-}$ mice increased their exploratory times with the objects in the trial with the novel object, apoE4 mice did not. ApoE4 mice required more trials than apoE3 or *Apoe*$^{-/-}$ mice to reach criterion during passive avoidance training, but castration did not modulate passive avoidance learning or memory. Thus, androgens have differential roles in object recognition and spatial learning and memory in the water maze, depending on whether or not apoE4 is present.

Theme: Neural basis of behavior
Topics: Learning and memory: physiology

Keywords: Androgen; Apolipoprotein E; Castration; Object recognition; Passive avoidance; Spatial learning and memory

1. Introduction

Distinct alleles (ε2, ε3, and ε4) encode the major human apolipoprotein (apo) E isoforms, which play important roles in the metabolism and redistribution of lipoproteins and cholesterol [23]. The three major human apoE isoforms, ε2, ε3, and ε4, are encoded by distinct alleles. In the brain, apoE has been implicated in development, regeneration, neurite outgrowth, and neuroprotection [4,23,28–30,32,39]. In addition, apoE has been implicated in the synthesis of glucocorticoids and sex hormones [42–44]. Compared with ε2 and ε3, ε4 increases the risk of cognitive impairments and of developing Alzheimer's disease (AD) [6]. Female sex is a risk factor for AD and apoE4 interacts with female sex, resulting in an even greater AD [41]. Consistent with these human data, adult apoE4 transgenic female, but not male, mice lacking mouse apoE (*Apoe*$^{-/-}$) show impairments in object recognition and spatial learning and memory in the water maze [31,33,34].

Sex steroids, which cause sex differences in brain organization and affect cognitive performance in adulthood, might contribute to the sexually dimorphic performance in

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learning and memory. Androgens have been shown to modulate hippocampal-dependent behavior, including learning and memory; testosterone enhanced spatial learning and memory [7,19,34–36] and both short-term and long-term emotional learning and memory [40]. In addition, post-training administration of androgens to ovariectomized rats enhanced spatial and emotional learning and memory temporally [8]. Finally, androgens modulated the expression of the immediate early gene c-*fos* in the hippocampus following exposure to a novel open field [21]. However, in some studies, androgens impaired spatial learning and memory [9,12–14] and administration of testosterone in the CA1 of the hippocampus [26] or the basolateral amygdala [27], brain areas with a high concentration of androgen receptors [18,38], impaired spatial learning and memory.

Androgens and androgen receptor-mediated signaling might protect against the detrimental effects of apoE4 on cognitive function. Brief periods of androgen treatment antagonized cognitive deficits in adult apoE4 female mice and acute AR blockade with hydroxyflutamide caused striking cognitive impairments in apoE4, but not apoE3, male mice [34]. In addition, expression of apoE4, but not apoE3, significantly reduced cytosolic AR binding in the neocortex compared to *Apoe*$^{-/-}$ mice. The lower circulating concentrations of endogenous androgens in females than males might contribute to their increased susceptibility to the detrimental effects of apoE4 on AR binding and cognitive function. To test this hypothesis, in the present study, we explored the influence of chronic reduced circulating androgens on the cognitive performance of human apoE transgenic male mice.

2. Materials and methods

2.1. Mice

The generation and genotyping of neuron-specific enolase (NSE)-apoE male mice have been described previously [31–34]. The *Apoe*$^{-/-}$ mice used in this study were littermate controls. To minimize the effects of social influences on behavior, 6–7-month-old mice (n = 45) were housed singly starting 1 week before behavioral testing under conditions of constant temperature (18°C), and light from 6:00 a.m. to 6:00 p.m. All mice had free access to food (PicoLab Rodent Diet 20, #5053, PMI Nutrition International, St. Louis, MO) and water.

2.2. Castration

Under isofluorane anesthesia, the skin of the scrotum of 3–4-month-old mice was opened. For mice receiving sham castration (*Apoe*$^{-/-}$: n = 9; apoE3: n = 5; apoE4: n = 8), the wound was than sutured. For mice receiving castration (*Apoe*$^{-/-}$: n = 9; apoE3: n = 6; apoE4: n = 8), the epididymis was cut and the testes removed. Subsequently, the wound was sutured. Behavioral testing started 3 months later.

2.3. Behavioral testing

The sequence of behavioral testing was such that tests were administered in the order of increasing stress level. The sequence of behavioral testing was: exploratory activity in the open field and rotorod, week 1; object recognition, week 2; spatial learning and memory in the water maze, week 3; and passive avoidance learning and memory, week 4. All animal experiments were carried out in accordance with the National Institute of Health guidelines for the care and use of Laboratory animals and approved by the Animal Care Committee at OHSU.

2.4. Open field

Since different levels of exploratory drive can affect motivation and performance in cognitive tests, exploratory behavior was assessed in the open field. Mice were placed singly in a brightly lit, automated infrared photocell activity arenas (40.64 × 40.64 cm with 16 × 16 photocells for measuring horizontal movements, 8 photocells for measuring rearing) interfaced with a computer (Hamilton-Kinder, Poway, CA). Active times (defined as time, within 1 s, in which a new beam was broken) and distance moved were calculated. Open field activity was recorded after a 1-min adaptation period for 10 min.

2.5. Rotorod

Rotorod balancing requires a variety of proprioceptive, vestibular, and fine-tuned motor abilities. The task requires the mouse to balance on a rotating rod with a 7.0 mm diameter (Hamilton-Kinder). After a 1-min adaptation period on the rod at rest, the rod was accelerated by 2 rpm every 30 s, and the length of time the mice remained on the rod (fall latency) was recorded. The mice received 9 trials, with a 25-min inter-trial interval.

2.6. Novel location and novel object recognition

After habituation to an open field, the mice were trained in three consecutive trials and than tested in two consecutive trials with a 5-min inter-trial interval. For both the training and testing sessions, three objects were placed in the open field, and the animal was allowed to explore for 10 min. All objects were only used once and replicas were used in subsequent trials. Five minutes after the training trials, the animals were tested for recognition of the novel location of one of the familiar objects. Five minutes after the novel location test, the animals were tested for novel object recognition. The time spent exploring each object during the training and testing sessions was recorded by an observer.